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A new highly troglomorphic species of *Eukoenenia* (Palpigradi: Eukoeneniidae) from tropical Brazil

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Abstract. A third troglobitic species of Brazil is described from three specimens collected within Gruta da Morena, located in the municipal district of Cordisburgo, Minas Gerais. *Eukoenenia sagarana* new species (Palpigradi: Eukoeneniidae) is highly adapted to subterranean environments, corresponding to the most troglomorphic Palpigradi species described to date. This new species is very close to *E. maquinensis* Souza & Ferreira 2010, a troglobitic species recently described from Gruta de Maquiné, located in the same municipal district. The differences and similarities between these two species and between *E. sagarana* and other species of *Eukoenenia* are presented.

Keywords: *Eukoenenia sagarana*, Neotropics, taxonomy, morphology

The order Palpigradi Thorell 1888 is considered one of the lesser-known groups within the Arachnida (Pepato et al. 2010). Fortunately, in recent years studies on this order have become more frequent (Barranco & Harvey 2008; Christian 2009; Christian et al. 2010; Souza & Ferreira 2010, 2011a, 2011b). However, data on Neotropical palpigrades are still very limited, and even more restricted for troglobitic Neotropical species, of which only three species have been described: *Eukoenenia orghidani* Condé & Juberthie 1981 from Cuba, *E. maquinensis* Souza & Ferreira 2010 from Brazil, and *E. spelunca* Souza & Ferreira 2010, also from Brazil.

The most distinetive troglomorphisms are found in species of the genus *Eukoenenia* Börner 1901. The morphological adaptations to the cave environment in this group consist mainly of increases in body size, elongation of the appendages (that also become thinner) and the increase in the number of sensory receptors, which correspond with the increase in elements that constitute the lateral organs (Condé 1988, 1998). According to Condé (1998), the length of basitarsus IV is the most reliable parameter to quantify the elongation of the appendages because, related to the length of the tibia, it provides an index of adaptation to the cave environment. In edaphic species, the tibia is longer than the basitarsus, forming a basitarsus:tibia ratio of less than one, while in troglobitic species the ratio will be close to, equal to, or higher than one. The value of the ratio among the lengths of the propeltidium and the basitarsus IV is also used to indicate possible levels of troglomorphism, and in edaphic species the value is approximately between three and four while in the cave species, it is less than two (Condé 1998).

The setae of the propeltidium, represented by ten pairs in most species, have a tendency to become smaller and sometimes less numerous in troglobites. A decrease in the number and size of the setae also occurs on the metapeltidium, with two pairs or one occurring instead of three (Condé 1992, 1998).

These troglomorphisms vary from each other in an independent way, according to the perceived evolutionary stage of the species (Condé 1998). Thus, for some species, certain structures will be more troglomorphic, while in others, more accentuated troglomorphisms can occur in different structures. Each species may acquire a series of unique characteristics due to its particular evolution in the subterranean environment, which makes it difficult to compare the species' degree of troglomorphism effectively. As a result, it is necessary for the group of characteristics to be analyzed as a whole and not each one in isolation.

In this work a new Brazilian species of *Eukoenenia* is described, which was collected in Gruta da Morena, near Cordisburgo, Minas Gerais. This new species is the second species of troglobitic palpigrade described from this municipal district and the third described from Brazil. Furthermore, it exhibits several notable troglomorphisms, and is considered here to be the most troglomorphic species of Palpigradi described to date.

METHODS

The specimens were examined by clearing in Nesbit's solution and mounting them in Hoyer's medium on 7.6 × 2.5-cm glass slides using the standard procedures developed for mites (Krantz & Walter 2009). All measurements are presented in micrometers (μm) and were taken using an ocular micrometer with a phase contrast microscope. We measured body length from the apex of the propeltidium to the posterior margin of the opisthosoma. The areoles in some drawings represent the insertions of setae.

Setal nomenclature and abbreviations follow Barranco & Mayoral (2007): L, total body length (without flagellum); B, dorsal shield length; P, pedipalpus; I and IV, legs I and IV; ti, tibia; bta1, basitarsus 1; bta2, basitarsus 2; bta3, basitarsus 3; bta4, basitarsus 4; ta1, tarsus 1; ta2, tarsus 2; ta3, tarsus 3; a, width of basitarsus IV at level of seta r; er, distance between base of basitarsus IV and insertion of seta r; grt, tergal seta length; gla, lateral seta length; r, stiff seta length; t/r, ratio between length of basitarsus IV and stiff seta length; t/er, ratio between basitarsus IV length and distance to insertion of stiff seta; gla/grt, ratio between lengths of lateral and tergal setae; B/bta, ratio between lengths of prosomal shield and basitarsus IV; bta/ti, ratio between lengths of basitarsus IV and tibia IV.

The specimens are lodged in the Coleção de Invertebrados Subterrâneos de Lavras, Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais (ISLA).

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TAXONOMY

Family Eukoeneniidae Petrunkevitch 1955
Genus *Eukoenenia* Börner 1901

Koenenia Grassi & Calandruccio 1885:165 [junior primary homonym of *Koenenia* Beushausen 1884 (Mollusca: Bivalvia)].

Koenenia (*Eukoenenia*) Börner 1901:551.

Type species.—*Koenenia mirabilis* Grassi & Calandruccio 1885, by monotypy.

Eukoenenia sagarana new species
Figs. 1–24

Material examined.—Male holotype, Brazil, Minas Gerais, Gruta da Morena cave (UTM 23 569484–7880316), Cordisburgo, 29 September 2010, R.L. Ferreira (ISLA 1409). Paratypes: 1 female, same data as holotype except 16 December 2009 (ISLA 1410); 1 juvenile, same data as holotype except 1 April 2010 (ISLA 1411).

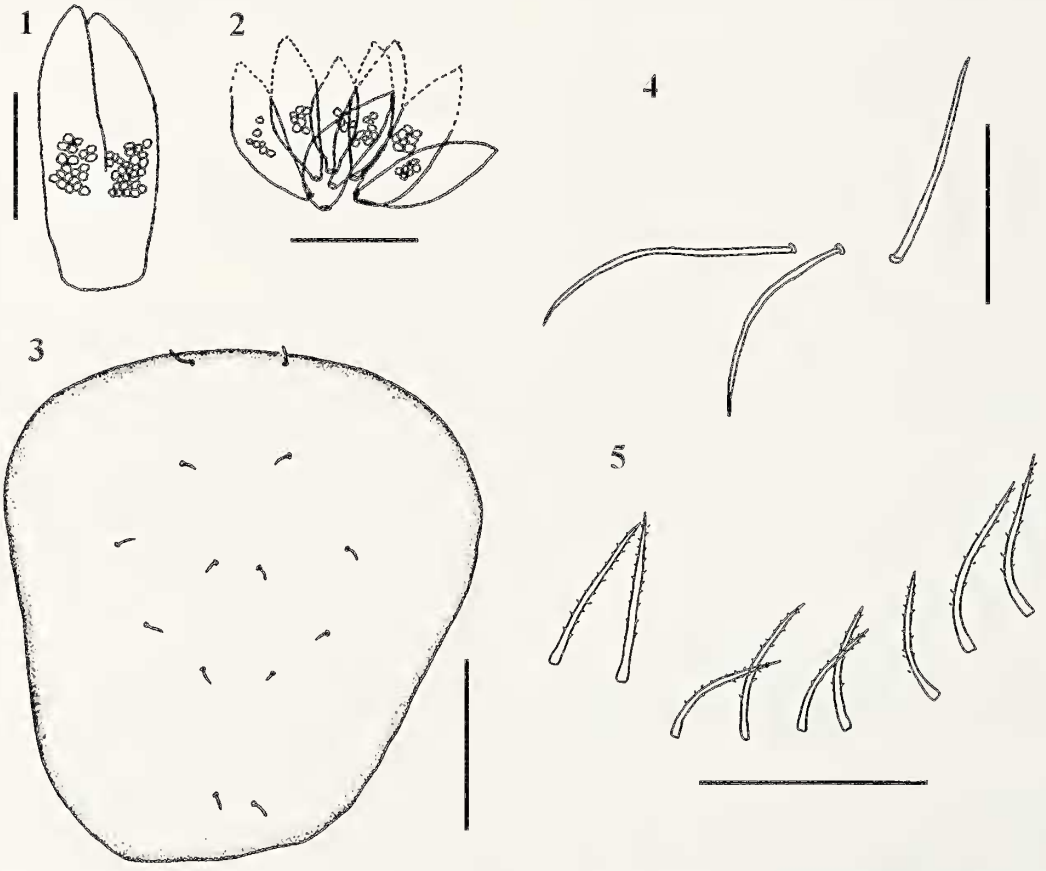
Diagnosis.—*Eukoenenia sagarana* differs from all other species of the genus by the following combination of characters: presence of 8–9 blades on prosomal lateral organs; propeltidium with 7 + 7 setae; six setae on basitarsus IV with a single proximal sternal seta; opisthosomal sternites IV–VI with 14, 14, and 13 setae, respectively, in the male and 15, 13 and 10 setae, respectively, in the female; and the singular shape of the spermatheca in the female genitalia.

Description of the adult stage.—Prosoma: frontal organ with two branches, blunt apically, each 5 times longer than wide (37.5 µm/7.5 µm) (Fig. 1). Lateral organ with eight pointed blades (nine blades in the male on the left side), each 3.25 times longer than wide (32.5 µm/10 µm) (Fig. 2). Propeltidium with 7 + 7 short setae, first pair on either side of frontal organ longer than others (Fig. 3). Metapeltidium with t_1, t_2, t_3 (157.5 µm, 125 µm and 117.5 µm) (Fig. 4). Deutotritosternum with nine setae in U-shaped arrangement (eight setae in the male) (Fig. 5).

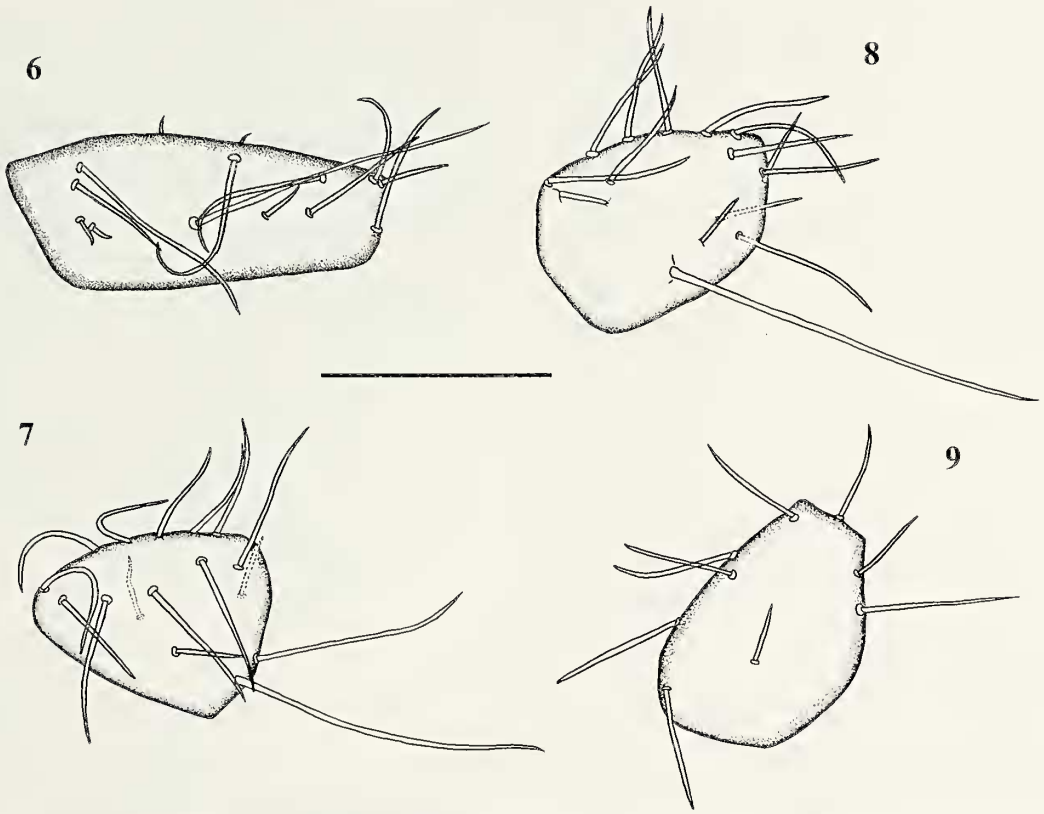
Coxal chaetotaxy: coxa I with 14 setae, coxa II with 6 thick and 10 normal setae, coxa III with 7 thick and 8 normal setae (5 thick and 9 normal setae in the male) and coxa IV with 2 thick and 7 normal setae (8 normal setae in the male) (Figs. 6–9).

Chelicerae with 9 teeth on each finger; 1 dorsal seta and 1 ventral seta inserted near the third segment, 1 seta inserted near the row of teeth of the second segment and a row with 3 setae inserted in a middle region (between the dorsal and ventral seta) (Fig. 10).

Basitarsus 3 of leg I slender, 4.7 times longer than wide, with 3 setae (grt 155 µm; r 132.5 µm). Seta r shorter than segment (152.5 µm/132.5 µm, t/r = 1.1), inserted in proximal half and surpassing hind edge (160 µm/45 µm, s/er = 3.5) (Fig. 11). Basitarsus of leg IV long, 11 times longer than wide, with 6 setae (2 esd, esp, gla, grt and r) (Fig. 12), bta/ti 1.02. Stiff seta r 2.7 times shorter than tergal edge of article



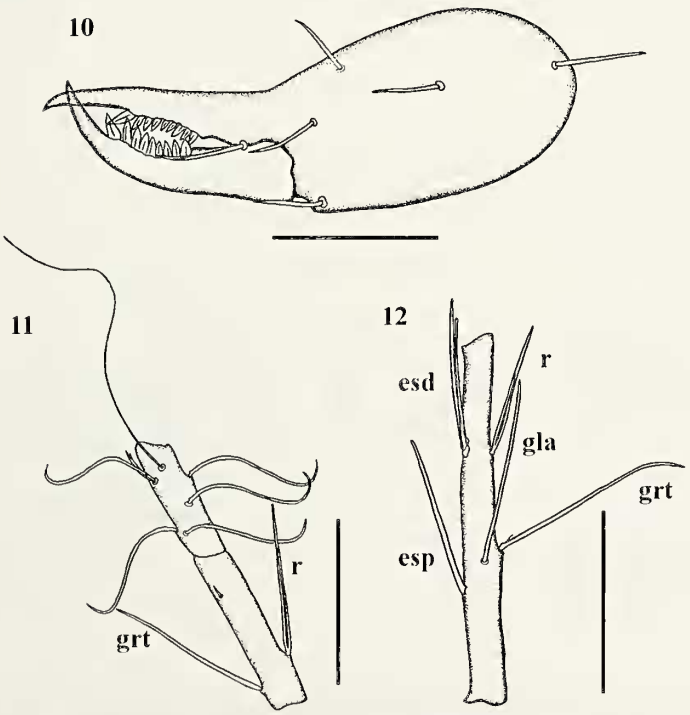
Figures 1–5.—*Eukoenenia sagarana* new species, female: 1. Frontal organ, dorsal view; 2. Lateral organ, dorsal view; 3. Propeltidial chaetotaxy; 4. Metapeltidial setae; 5. Deutotritosternal setae. Scale bars 20 µm (Fig. 1), 20 µm (Fig. 2), 150 µm (Fig. 3), 100 µm (Fig. 4), 60 µm (Fig. 5).



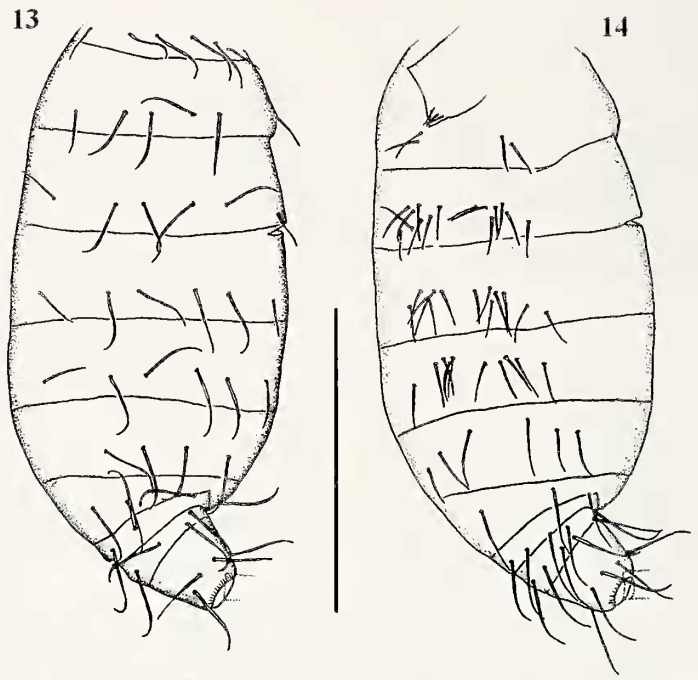
Figures 6–9.—*Eukoenenia sagarana* new species, female: 6. Coxa I; 7. Coxa II; 8. Coxa III; 9. Coxa IV. Scale bar 150 μm.

(302.5 μm/112 μm, t/r = 2.7) and inserted in distal third (302.5μm/187.5 μm, t/er = 1.61). Seta esp proximally inserted, followed by gla and grt, more or less at the same level, all of them in proximal half.

Opisthosoma: female: tergites II–VI with 3 + 3 setae each, 2 pairs of tergal setae (t_1, t_3) between both slender setae (s) (Fig. 13). Sternite III with 2 + 2 setae. Sternite IV with 15 setae (13 thickened setae between both slender setae); sternite V



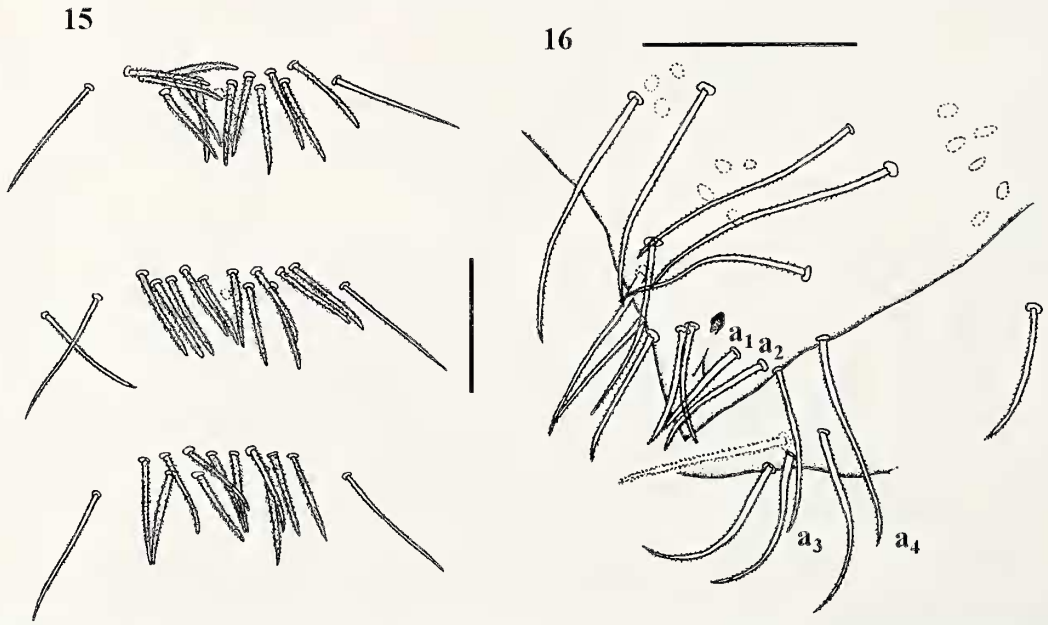
Figures 10–12.—*Eukoenenia sagarana* new species, female: 10. Chelicera; 11. Basitarsus 3–4 of leg I; 12. Basitarsus IV. Scale bars 150 μm.



Figures 13,14.—*Eukoenenia sagarana* new species, female: 13. Opisthosoma, dorsal view; 14. Opisthosoma, ventral view. Scale bar 600 μm.

with 13 setae (11 thickened setae between both slender setae); sternite VI with 10 setae (8 thickened setae between both slender setae). Sternites IV–VI each with one glandular pore on the median line. Segments VII–XI each with 8, 10, 9, 9 and 8 setae, respectively (Fig. 14). Male: tergites II–VI and sternite III as in female. Sternite IV with 14 setae (12 thickened setae between both slender setae); sternite V with 14 setae (11 thickened setae between two slender setae on one side and one on the other); sternite VI with 13 setae (11 thickened setae between both slender setae). Setae of the a-groups considerably thicker and subcylindrical than that found in the female. Sternites IV–V each with two glandular pores on the median line (Fig. 15). Segments VII–XI eaeh with 8, 9, 8, 8 and 8 setae, respectively.

Female genitalia: first lobe with 11 + 12 setae in 5 transverse rows, 4 sternal 2 + 2, 2 + 3 (asymmetry caused by disloeations due to the lack of regular and/or the presence of additional setae), 2 + 2, 1 + 1 and distal 4 + 4, of which a_1, a_2, a_3, a_4 measure 30–32.5 μm, 35–40 μm, 42.5–45 μm and 57.5 μm, respectively. Second lobe with 3 + 3 setae (x, y, z) (second lobe is curved doubled bent), measuring 40 μm, 55 μm and 42.5 μm, respeetively; 13 glandular orifices (Fig. 16). Spermatheca illustrated in Fig. 16.



Figures 15,16.—*Eukoenenia sagarana* new species: 15. Opisthosomal sternites IV–VI of male; 16. Female genitalia. Scale bars 100 μm (Fig. 15), 60 μm (Fig. 16).

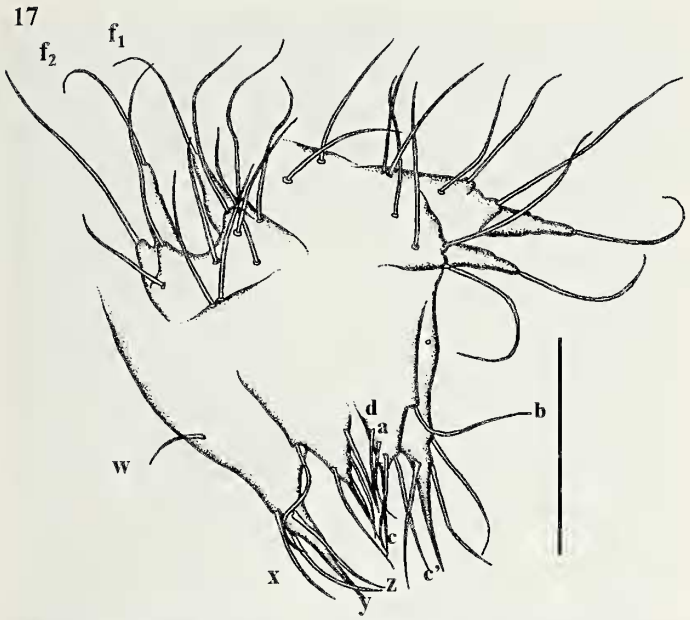
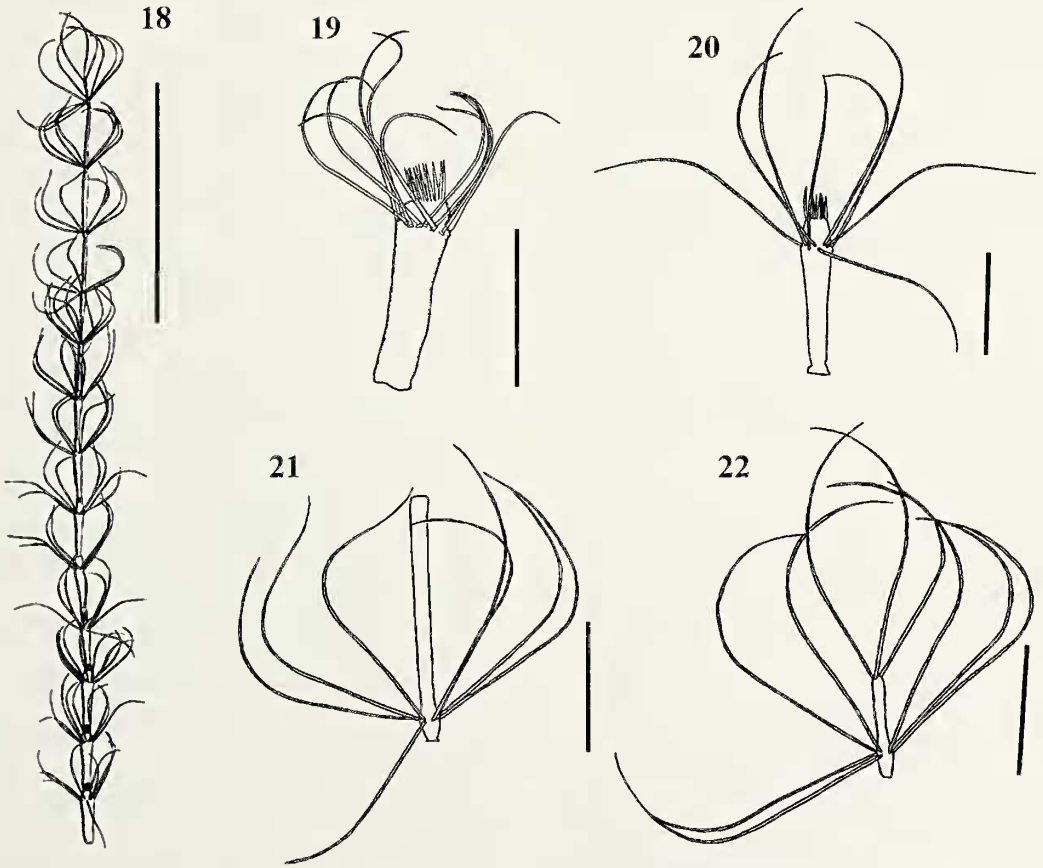


Figure 17.—*Eukoenenia sagarana* new species, holotype: male genitalia. Scale bar 150 μm.

Male genitalia: first lobe appears as two elements, wider than long, with 13 + 13 setae (including 2+2 fusules in the distal margin); f_1 = 150–152.5 μm; f_2 = 140–145 μm. Second lobe subtriangular, with a simple and sharp apex (without bifurcation), with 5 + 5 setae (a, b, c, c', d). Third lobe also in a subtriangular form, well developed, with 4 + 4 setae (w, x, y, z), with a large, sharp and simple acute apical region (Fig. 17).

Flagellum: longer than body length, with 14 long, slender segments (Fig. 18). Segments 1, 3, 5, 7 and 9 with an apical crown of thorns. Segments 1–9 with 12, 9, 11, 11, 8, 10, 8, 10 and 8 long setae, respectively, inserted in the distal half (Figs. 19–20). Tenth segment with 8 long setae inserted in the distal half, but closer to the middle of the segment. Eleventh segment with 8 long setae in proximal half (Fig. 21). Twelfth and thirteenth segments with 8 long setae inserted in proximal third. Last segment with 7 long setae inserted in proximal third of segment and 3 setae inserted apically (Fig. 22).

Description of juvenile female (larve B *sensu* Condé 1996): Frontal organ with two branches, each 5 times longer than wide (25 μm/5 μm). Lateral organ with seven blades, each 4.25 times longer than wide (42.5 μm/10 μm). Deutotritosternum with 6 setae. Fingers of chelicera with 8 teeth. Chaetotaxy of propeltidium and metapeltidium complete. Coxal chaetotaxy: coxa I with 13 setae, coxa II with 4 thick and 10 normal setae, coxa III with 4 thick and 10 normal setae and coxa IV with 1 thick and 8 normal setae. Trichobothria and forked setae as in adult. IV bta and opisthosomal tergites II–VI similar to the adult. Sternites IV–VI with 13, 14 and 14 setae, respectively. Segments VII–XI with 10, 8, 8, 8 and 8 setae. Primordia of genital lobes developed on segments II and III. Segment II with 6 + 6 setae and segment III with 1 + 1 setae (Fig. 23). The



Figures 18–22.—*Eukoenenia sagarana* new species, female: 18. Flagellum (the first segment is lacking); 19. First flagellar segment; 20. Fifth flagellar segment; 21. Eleventh flagellar segment; 22. Last flagellar segment. Scale bar 1300 μm (Fig. 18), 200 μm (Figs. 19–22).

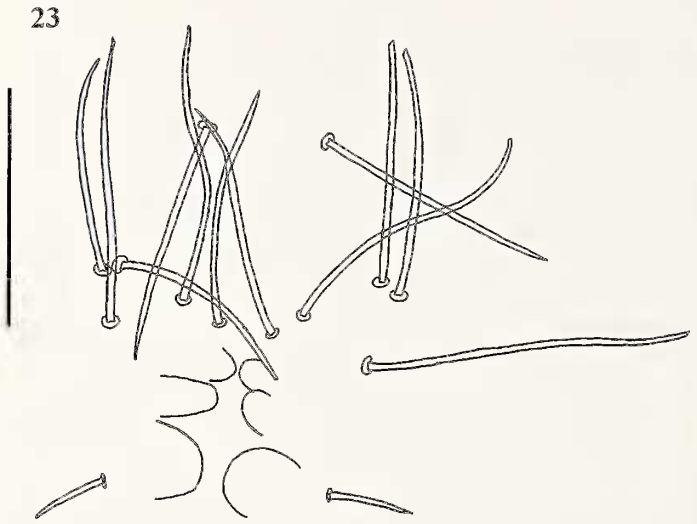


Figure 23.—*Eukoenenia sagarana* new species, juvenile female: primordia of genital lobes. Scale bar 60 μm.

habitus of the immature specimen (without the flagellum) is shown in Fig. 24.

Morphometric data are given in Table 1.

Etymology.—The specific name refers to *Sagarana*, a book of stories by the famous Brazilian writer João Guimarães Rosa, born in Cordisburgo. *Sagarana* is formed by a hybridism: "*saga,*" radical of Germanic origin that means "legend"; and "*rana*" word of Tupi origin (Brazilian Indian language), that means "that which expresses similarity." Therefore, *Sagarana* means "close to a legend." It is to be treated as a noun in apposition.

DISCUSSION

Sexual dimorphism in the Palpigradi is quite conspicuous in *Koeneniodes* Silvestri 1913 and *Prokoenenia* Börner 1901 and more discrete in the other genera. It arises mainly from the glandular complexes and setae located on sternites IV–VI (Condé 1991). In *E. sagarana,* differences can be observed in the number and form of the thickened setae, since the male has more numerous and considerably thicker and more cylindrical setae than the female. Similar differences can be observed in other species of the genus *Eukoenenia* such as *E. janetscheki* Condé 1993, *E. tetraplumata* Moreno 2007 and *E. maroccana* Barranco & Mayoral 2007 (Condé 1993, 1997; Moreno 2006; Barranco & Mayoral 2007).

Other species of the genus *Eukoenenia* also have numerous setae **a** on the opisthosomal sternites: *E. pretneri* Condé 1977 (5a + 5a on sternite IV, 6a + 6a on sternite V and 1a + 1a on sternite IV), *E. bouilloni* Condé 1980 (sternites IV–VI with 10, 9 and 7 thick setae, respectively) and *E. bonadonai* Condé 1979 (sternites IV–VI with two regions of thick setae, approximately symmetrical and contiguous including 11 + 12 bristles on IV, 14 + 13 on V and 12 + 13 on VI). In these species, however, it is not known if there is sexual dimorphism in the number of thick setae on the opisthosoma, as only the males are known. The female of *E. maquinensis* also has numerous setae on sternites IV–VI (14, 13 and 11 respectively), having little differentiation between thickened setae (a) and normal setae (s) located in these segments (Souza and Ferreira 2010), which also occurs in the female of *E. sagarana.*

Figure 24.—*Eukoenenia sagarana* new species, juvenile female: habitus, dorsal view.

The same asymmetry present in the genitalia of the female holotype of *E. maquinensis* (12 + 11 setae on the first lobe) is also observed in *E. sagarana.* The male genitalia have a unique form and chaetotaxy, with relatively long and prominent fusules.

Eukoenenia sagarana is a troglobitic species very similar to *E. maquinensis,* the first described troglobitic species from South America (Souza and Ferreira 2010). The first species occurs in the Gruta da Morena cave and the second in the Gruta de Maquiné, both located in the municipal district of Cordisburgo (Minas Gerais), and only about 5 km from each other. However, a stream crosses between the two caves, which may impede sub-surface migration of organisms between these systems. These two species share many important taxonomic characteristics, mainly being distinguished by the number of elements that form the lateral organs, the disposition of the setae in the chelicerae, the number of setae of the abdominal sternites IV–VI, and the form of the spermatheca.

Both show quite accentuated troglomorphisms, as females of both species have a bta VI/Ti ratio equal to 1.07 (1.02 in the male and 0.94 in the juvenile of *E. sagarana*). However, *E. sagarana* also has a higher degree of adaptation to the cave environment when compared not only to *E. maquinensis,* but also to the highly troglomorphic Greek species *E. naxos* Condé 1989, for having a B/btaIV ratio of 1.43, a value even lower than that presented by these two species (1.58–1.7 in *E. maquinensis* and 1.71 in *E. naxos*) (Condé 1989; Souza & Ferreira 2010). Also, the new species has an extremely

Table 1.—Measurements (μm) of selected body parts of the specimens of *Eukoenenia sagarana* new species.

Body part	Male (holotype)	Female (paratype)	Juvenile female
L	2020	1900	1595
B	435	420	352.5
Pti	262.5	242.5	190
Pbta1	102.5	87.5	72.5
Pbta2	117.5	112.5	85
Pta1	57.5	55	45
Pta2	82.5	72.5	60
Pta3	100	92.5	77.5
Iti	-	327.5	-
Ibta1+2	285	252.5	-
Ibta3	152.5	147.5	105
Ibta4	130	120	90
Ita1	72.5	72.5	55
Ita2	67.5	67.5	62.5
Ita3	222.5	232.5	190
IVti	295	272.5	222.5
IVbta	302	292.5	210
IVta1	107.5	97.5	80
IVta2	137.5	125	110
A	27.5	25	25
Er	187.5	197.5	145
Grt	192.5	160	127.5
Gla	155	150	105
R	112	102.5	77.5
tlr	2.7	2.85	2.7
tler	1.61	1.48	1.44
gla/grt	0.8	0.93	0.82
B/btaIV	1.43	1.43	1.67
btaIV/tiIV	1.02	1.07	0.94
FI	237.5	220	-
FII	280	-	-
FIII	305	-	-
FIV	330	-	-
FV	280	-	-
FVI	345	-	-
FVII	282.5	-	-
FVIII	345	-	-
FIX	272.5	-	-
FX	370	-	-
FXI	430	-	-
FXII	360	-	-
FXIII	355	-	-
FXIV	150	-	-

elongated basitarsus IV, being 11.7 times longer than wide in the female. This value is 8.8 in *E. maquinensis* and 10.22 in *E. naxos*. The number of elements that constitute the lateral organ in *E. sagarana* (8–9) is larger than that observed in those two species (6 and 5 respectively). The presence of 8 elements forming the lateral organ is an uncommon characteristic (as shown in the female of *E. sagarana*), being shared with only five species of *Eukoenenia*: *E. grafittii* Condé & Heurtault 1993 (8), *E. lyrifer* Condé 1992 (8), *E. patrizii* (Condé 1956) (8–10), *E. draco* (Peyerimhoff 1906) (8) and *E. hispanica* (Peyerimhoff 1908) (8). Furthermore, it is important to point out that Souza & Ferreira (2010) had considered *E. maquinensis* as the species with the longest known flagellum (3.865 mm). *Eukoenenia sagarana*, in turn, has the longest flagellum described to date, with a length of 4.3 mm.

When comparing the many characteristics shared by *E. sagarana* and *E. maquinensis*, it is plausible to assume that these two species originated from a common ancestor. Probably this one ancestor took shelter in hypogean habitats during drastic climatic changes, withstanding isolation and forming these two species that remain restricted to the Gruta da Morena and Gruta de Maquiné caves. Due to the absence of efficient external inventories, it is difficult to determine if this ancestral surface lineage still exists or is locally extinct. What can be affirmed is that this isolation occurred a long time ago, keeping in mind the high degree of adaptation to the subterranean environment presented by these species. In some caves of the area (including the Gruta da Morena), as well as in some epigean habitats, edaphomorphic Palpigradi have been found, probably belonging to the species *E. florenciae* (Rucker 1903). However, there is no indication that *E. maquinensis* and *E. sagarana* have any phylogenetic relationship with this species, since they do not share any morphological characteristics. Therefore, it is possible that *E. florenciae* colonized the area after the speciation event that gave rise to the troglobitic species.

Eukoenenia naxos was considered by Condé (1998) to be the species that reached the highest degree of subterranean evolution for a range of characteristics, except for the number of lateral organs. Among the various troglomorphisms shown by this species, the substitution of the tergal seta of basitarsus IV by a trichobothrium can be mentioned and the largest bta IV/ti ratio observed for a type of Palpigradi. However, the great elongation of the appendages evidenced by the B/bta IV ratio and by the ratio between the length and width of bta IV, the presence of 8–9 blades forming the lateral organs and the extreme elongation of the flagellomeres suggests *E. sagarana* has more troglomorphic characteristics than *E. naxos*. Therefore, this new species can be considered the palpigrade species most adapted to the subterranean environment described to date. This fact reinforces the idea presented by Souza & Ferreira (2010) that the effects of climatic changes in the Neotropical region were similar to the temperate region on some groups, such as the palpigrades.

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